GeneProof®

PCR Kit

This manual is designed for the following kits:

Mycobacterium tuberculosis PCR kit, *Borrelia burgdorferi* PCR kit, *Chlamydia trachomatis* PCR kit, *Chlamydia pneumoniae* PCR kit, *Neisseria gonorrhoeae* PCR kit, *Mycoplasma pneumoniae* PCR kit

CE

***in vitro* Diagnostics**

Manual for use with the following device

SmartCycler®

VERZE K1-VERZE01-CZ

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GeneProof PCR kit

GeneProof PCR kits, designed for the detection and quantification of pathogen DNA, are based on the principle of amplifying specific target sequences of microorganisms and measuring the amplification product concentration growth in the course of the polymerase chain reaction by means of fluorescence-marked probes (the probe designated for pathogen detection is marked by the FAM fluorophore. The reaction mix includes an Internal Standard (IS) controlling the possible inhibition of the PCR reaction and the efficiency of DNA isolation process. Amplification of IS results in positive signal in TET channel.

GeneProof PCR kits

- Use the "hot start" technology, minimizing non-specific reactions and assuring maximum sensitivity.
- Contain uracil-DNA-glycosylase (UDG), controlling possible contamination of the PCR reaction by amplification products.
- All PCR kits for pathogen DNA detection can be amplified by means of a universal amplification program.
- Are easy to use; the kits always contain one tube with MasterMix and one tube with Positive Control (or with an Internal Standard) or a set of calibration controls.
- Are designed for in vitro diagnostics (CE IVD certification)

ISIN and ISEX versions of the GeneProof PCR Kit

All GeneProof PCR kits include an Internal Standard providing for an effective monitoring of eventual inhibition of the PCR amplification and also of the isolation process efficiency. The Internal Standard is a precisely defined and quantified construct of a plasmid and insert, prepared by methods of genetic engineering. **GeneProof develops and sells two basic versions of PCR kits with various compositions of the Internal Standard:**

PCR kit ISIN (Cat. No. *PCR kit*/ISIN/)

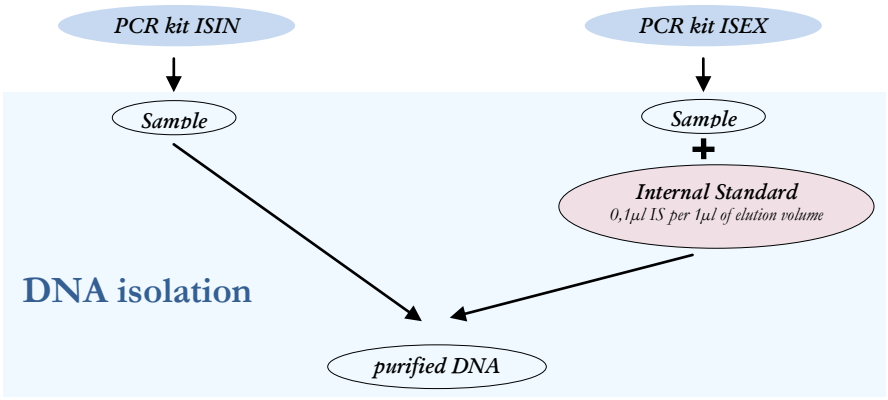
In this version of the PCR kit the Internal Standard is included in the MasterMix tube. This PCR kit version enables PCR inhibition control.

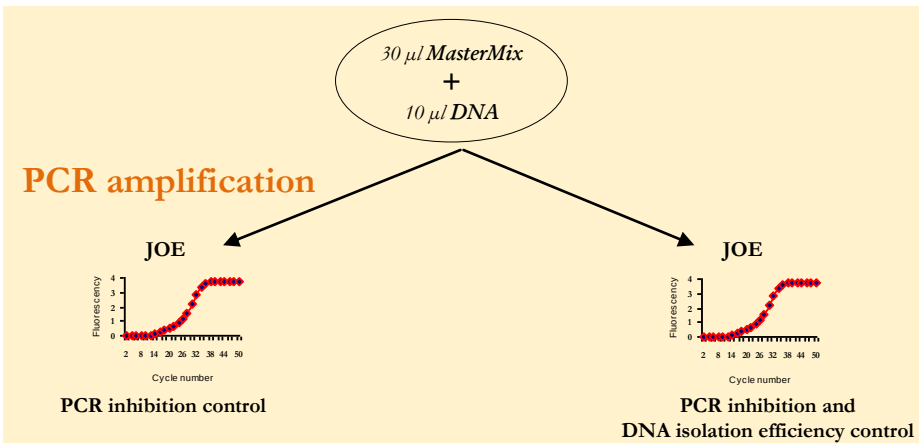
PCR kit ISEX (Cat. No. *PCR kit*/ISEX/)

In this PCR kit version the Internal Standard is included as an independent item within the package. This PCR kit enables both, PCR inhibition control and DNA isolation process efficiency control.

The Internal Standard should be added into the sample at the beginning of the isolation process as follows: 0.1 µl of the Internal Standard per 1 µl of elution volume:

Elution Volume	25 µl	50 µl	100 µl	200 µl
Internal Standard	2.5 µl	5 µl	10 µl	20 µl



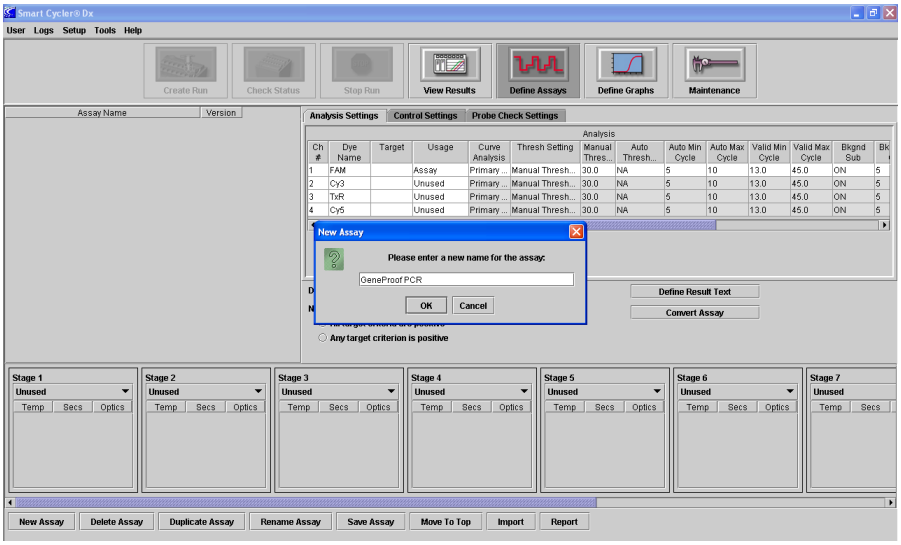


Reaction preparation and device programming

Add **15 µl of the MasterMix** and **10 µl of the DNA isolate** or **10 µl of the Positive Control** into a SmartCycler tube. The final reaction mix volume will be 25 µl. Close the tubes and centrifuge them shortly. Insert them into the device and program according to the following procedure:

1. Start the **SmartCycler® Dx** software, select **Define Assays** tab in the main menu and select New Assay in the bottom menu. **New Assay** window opens. Type in **GeneProof PCR** and click **OK** (Fig. 1.).

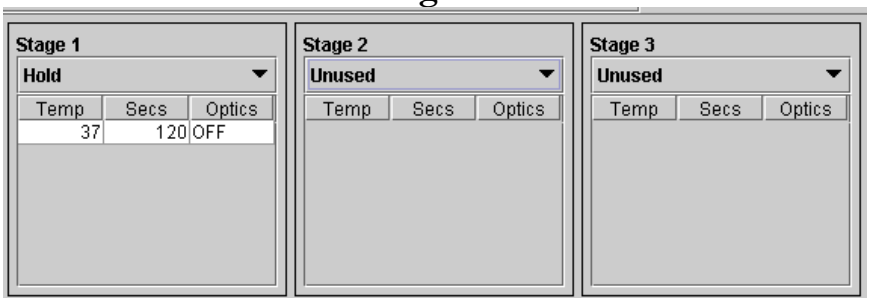
Fig. 1. Creating a new PCR program.



2. Program the PCR temperature profile according to following instructions:

   Step 1:
   In the **Stage 1** window select **Hold** from the drop-down menu, type **37** in the **Temp** field, type **120** into the **Secs** field and select **OFF** in the **Optics** column (Fig. 2.).

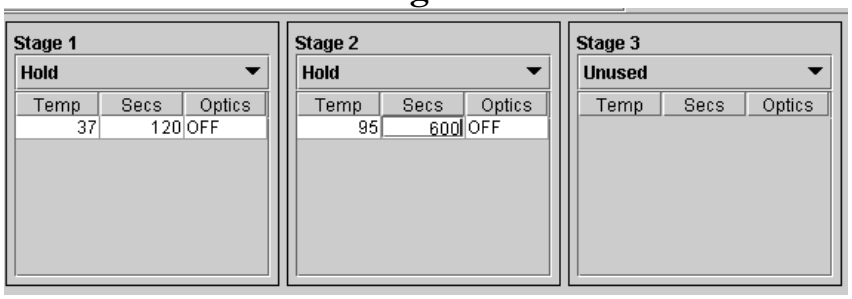
Fig. 2.



Step 2:

In the **Stage 2** window select **Hold** from the drop-down menu, type **95** in the **Temp** field, type **600** into the **Secs** field and select **OFF** in the **Optics** column (Fig. 3.).

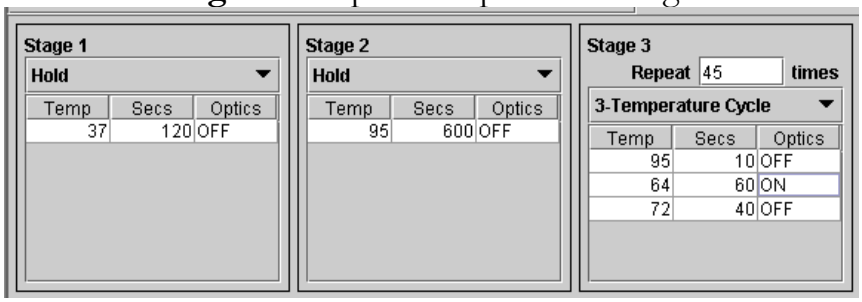
Fig. 3.



Step 3:

In the **Stage 3** window select **3-Temperature Cycle** from the drop-down menu. Type **45** in the **Repeat** field. Type **Temp 95, Secs 10** and **Optics OFF** into the first line, type **Temp 64, Secs 60** and **Optics ON** into the second line, type **Temp 72, Secs 40** and **Optics OFF** into the third line (Fig. 4.).

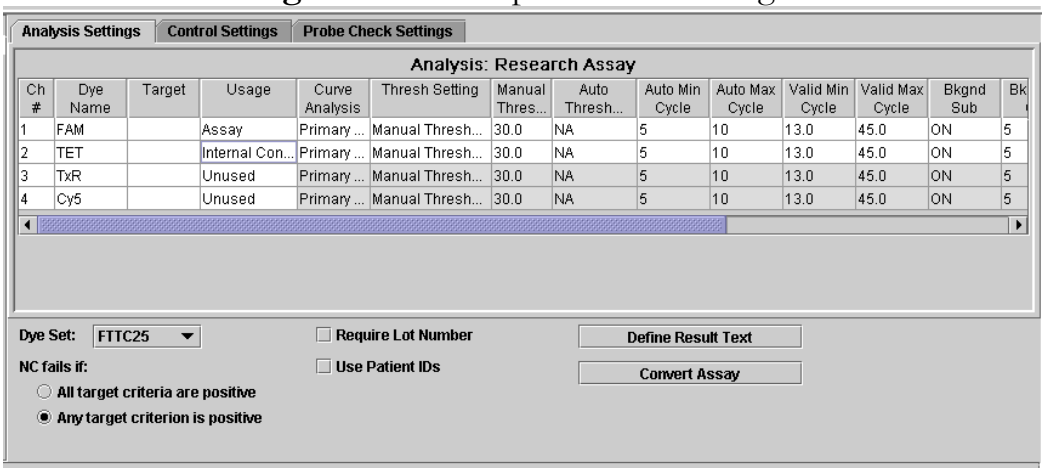
Fig. 4. Temperature profile setting.



3. Set the fluorophore combination in the **Dye Set** item as **FTTC25**. At the **NC fails if** item check **Any target criterion is positive** (Fig. 5.).

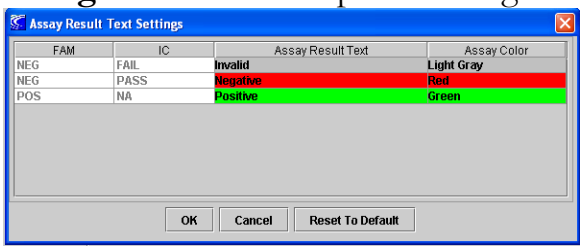
4. In the **Analysis Settings** tab choose **Assay** in the **Usage** column for channel 1 (FAM). In the **Thresh Settings** column choose **Manual Threshold**, type **30** into the **Manual Threshold** column field. Enter **ON** in the **Bkgnd Sub** column, this parameter applies automatically on all used channels. For channel 2 (TET) choose **Internal Control** in the **Usage** column. In the **Thresh Settings** column choose **Manual Threshold**, type **30** into the **Manual Threshold** column field. For channels 3 and 4 (TxR and Cy5) choose **Unused** in the **Usage** column. Leave all other parameters unchanged (Fig. 5.).

Fig. 5. Detection parameters settings.


Analysis Settings
Control Settings
Probe Check Settings
Analysis: Research Assay
Ch # | Dye Name | Target | Usage | Curve Analysis | Thresh Setting | Manual Thres... | Auto Thresh... | Auto Min Cycle | Auto Max Cycle | Valid Min Cycle | Valid Max Cycle | Bkgnd Sub | Bk
1 | FAM | | Assay | Primary ... | Manual Thresh... | 30.0 | NA | 5 | 10 | 13.0 | 45.0 | ON | 5
2 | TET | | Internal Con... | Primary ... | Manual Thresh... | 30.0 | NA | 5 | 10 | 13.0 | 45.0 | ON | 5
3 | TxR | | Unused | Primary ... | Manual Thresh... | 30.0 | NA | 5 | 10 | 13.0 | 45.0 | ON | 5
4 | Cy5 | | Unused | Primary ... | Manual Thresh... | 30.0 | NA | 5 | 10 | 13.0 | 45.0 | ON | 5
Dye Set: FTTC25
Require Lot Number
Define Result Text
NC fails if:
Use Patient IDs
Convert Assay
All target criteria are positive
Any target criterion is positive

5. Description of the results can be set by opening of the **Define Result Text** button. Positive result has the **POS** parameter in the **FAM** column and **NA** parameter in the **IC** column. Negative result has **NEG** parameter in the **FAM** column and **PASS** parameter in the **IC** column. In case of an invalid result the sample has **NEG** in the **FAM** column and **FAIL** in the **IC** column. (Fig. 6.).

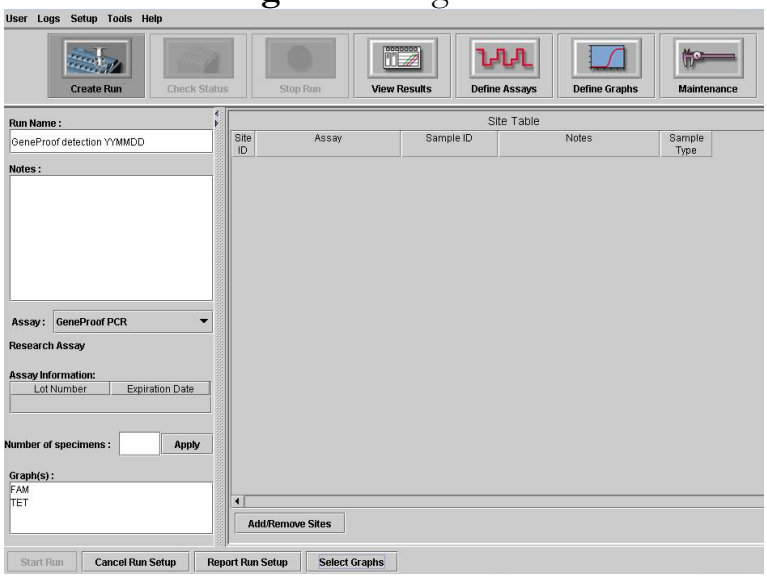
Fig. 6. Result description settings.


Assay Result Text Settings
FAM | IC | Assay Result Text | Assay Color
NEG | FAIL | Invalid | Light Gray
NEG | PASS | Negative | Red
POS | NA | Positive | Green
OK
Cancel
Reset To Default

6. Save the created program using the **Save Assay** button in the bottom menu.

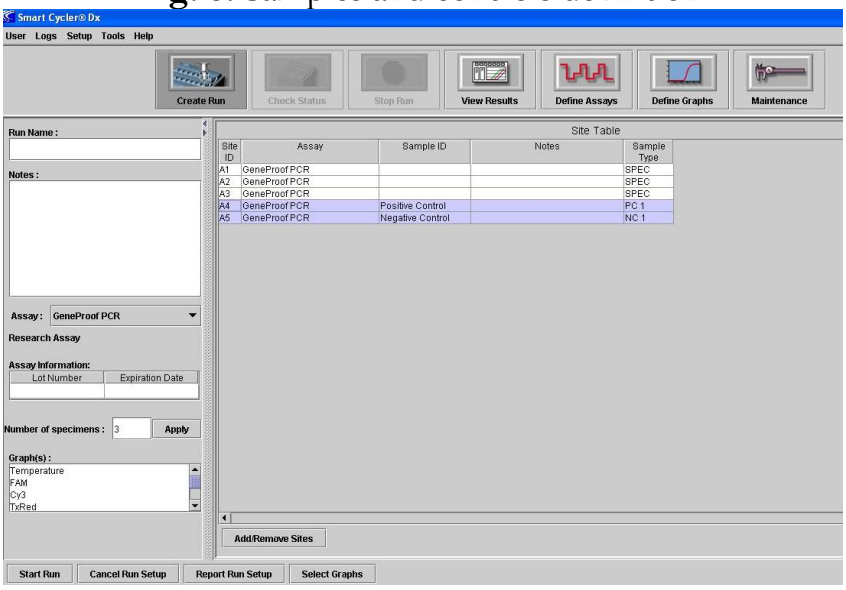
7. To start a run choose **Create Run** tab in the main menu. Enter the name of the detection kit used in the **Run Name** field. Choose GeneProof PCR program created in the first part of this manual in the **Assay** drop-down menu. (Fig. 7.).

Fig. 7. Starting a run.



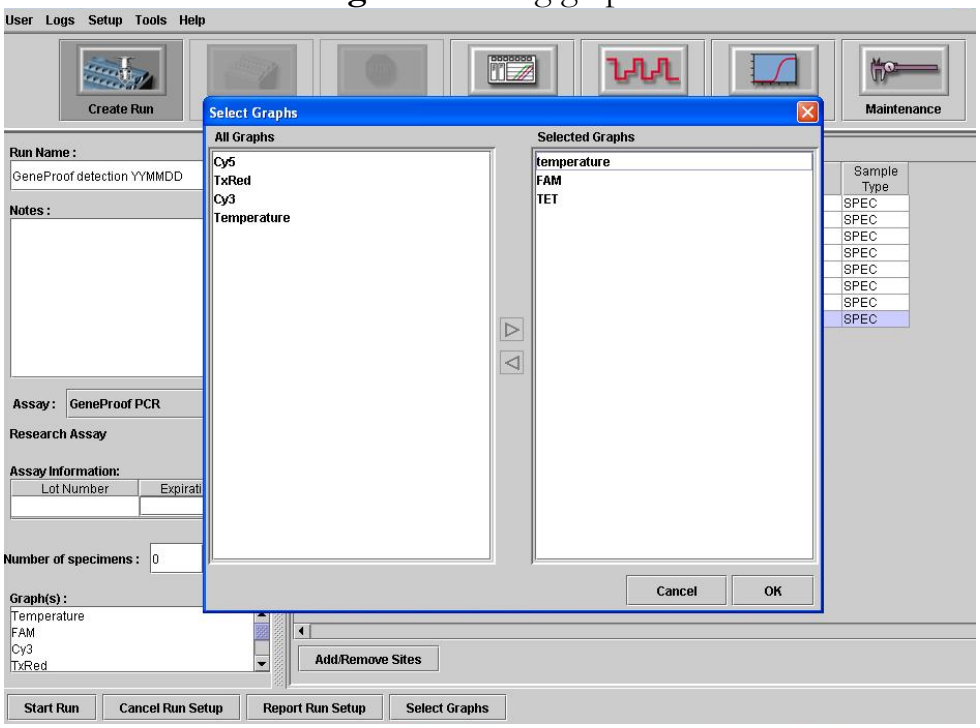
8. Type the number of samples to be examined into the **Number of specimens** field and click **Apply** (count in only the samples without external controls like Positive and Negative control. These will be automatically included by the software after the samples to be examined.) The final number of specimens and their order will be shown in the main window. The number of specimen and their order can be modified if necessary by selecting the **Add/Remove Sites** button below the main window. Use the arrows to add or remove sample positions. In case of redefining the positive or negative control position, select **PC1** (for positive control), **NC1** (for negative control) or **SPEC** (for unknown sample) in the **Sample Type** column in the main window. Enter sample description into the **Sample ID** column for better orientation (Fig. 8.).

Fig. 8. Samples and controls definition.



9. Real-time imaging of selected values can be set by selecting the **Select Graphs** button in the bottom menu. Use the arrows to select **FAM, TET** and **temperature** and confirm **OK**. The graph parameters can be changed also in the course of the PCR run after starting the machine (Fig. 9.).

Fig. 9. Selecting graphs.

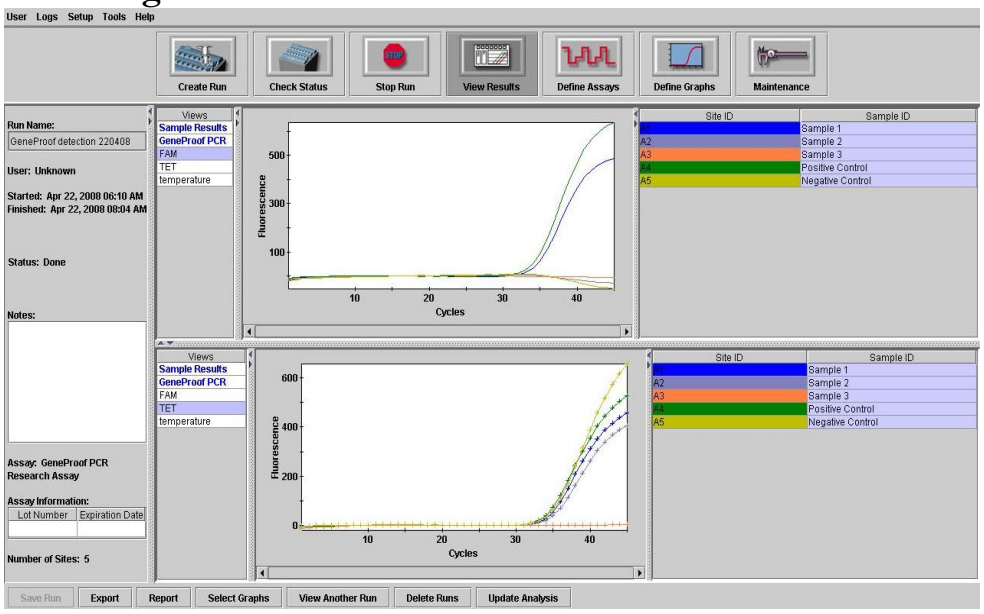


10. Start the examination by selecting the **Start Run** button.

Detection evaluation

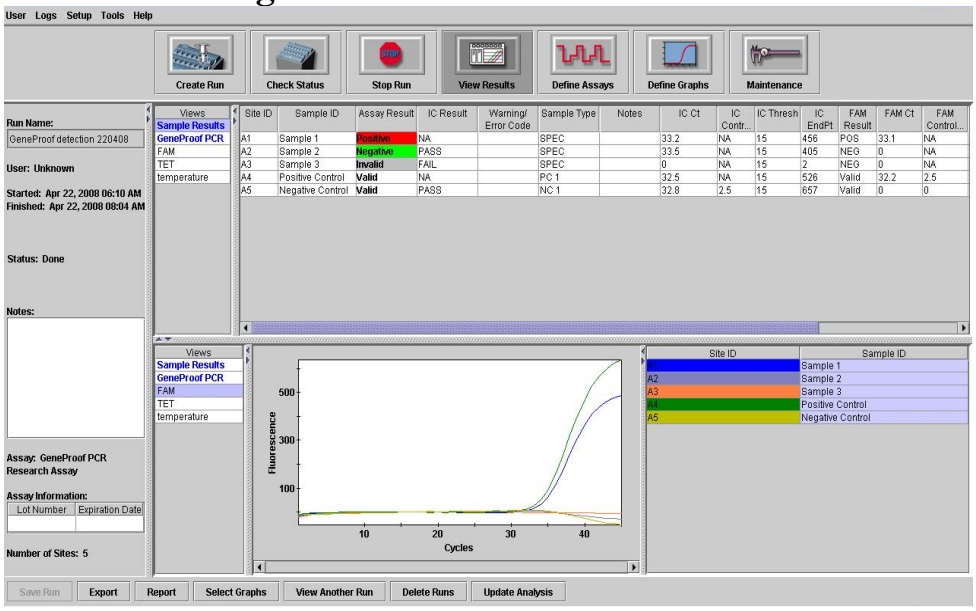
1. After the program finishes select **View results** tab in the main menu. In the Views window select the FAM channel for positive signal control and TET channel for internal standard detection control (Fig. 10.).

Fig. 10. Results view in the FAM and TET channels.

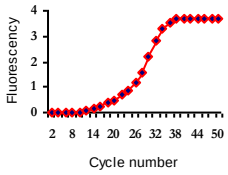
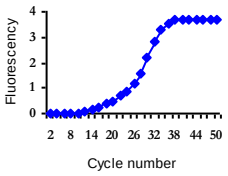
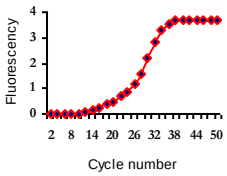
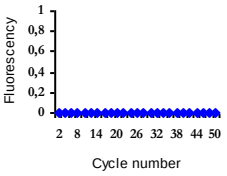
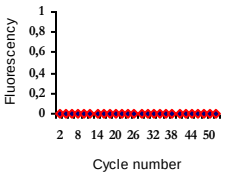
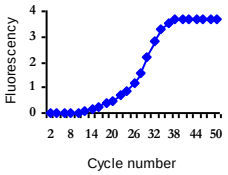
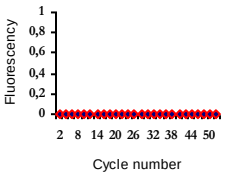
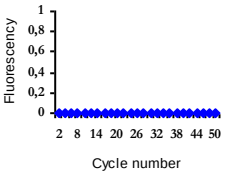


2. For evaluating the results select **Sample Results** in the **Views** window. An automatic evaluation of the results according to preset parameters shows in the **Assay Result** column. (Fig. 11.).

Fig. 11. Detection results evaluation.



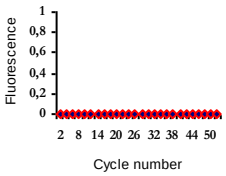
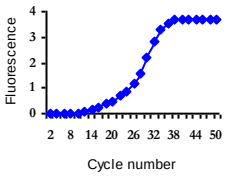
Detection evaluation:

FAM	TET	Result
		**Positive**
		**Positive**
		**Negative**
		**Invalid result ***

* see Troubleshooting p. 12

Troubleshooting

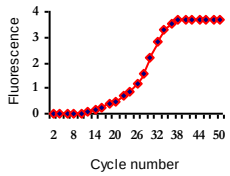
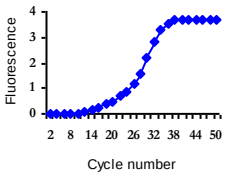
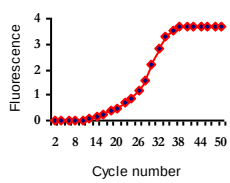
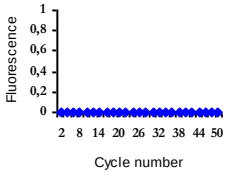
Invalid result of a Positive Control analysis

FAM	TET	Result
		?

- Problem: *Incorrect programming of the PCR amplification*
  Problem solution:
  1. Check device programming according to the manual
  2. Check correct temperature settings in the individual program blocks

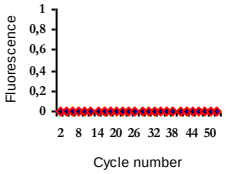
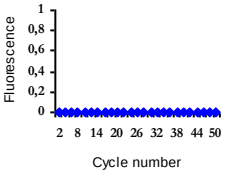
- Problem: *Positive control incorrectly held in storage* (see Storage and transportation conditions)
  Problem solution:
  1. Check whether kit component is stored according to manufacturer's recommendations
  2. Submultiple the Positive control and do not freeze and thaw it

Invalid result of a Negative Control analysis

FAM	TET	Result
		?
		?

- Problem: *PCR reaction contamination*
  Problem solution:
  1. Check the process of preparation and pipetting of the PCR mix into tubes
  2. Check whether sterile plastics and filtered tips are used
  3. Clean the PCR box
  4. Ad uracil-DNA-glycosylase (UDG) into the reaction

Invalid result of an Unknown Sample analysis

FAM	TET	Result
		?

- Problem: *PCR reaction inhibition* (PCR kit ISIN and ISEX)
  Problem solution:
  1. Repeat DNA isolation
  2. Check the process of preparation and pipetting of the PCR mix into tubes

- Problem: *Invalid process of DNA isolation* (PCR kit ISEX)
  Problem solution:
  1. Repeat DNA isolation
  2. Check the process of preparation and pipetting of the Internal Standard at the beginning of the isolation process.

- Problem: *Incorrect storage of the MasterMix* (see Storage and transportation conditions)
  Problem solution:
  3. Check whether MasterMix is stored according to manufacturer's recommendations
  1. Submultiple the MasterMix and do not freeze and thaw it

If you have any questions please contact our Product Support Department at: **support@geneproof.com**

Notes: